International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN : 0974-4290
Vol.6, No.12, pp 5142-5145, October 2014

CBSE-2014 [2nd and 3rd April 2014]

Challenges in Biochemical Engineering and Biotechnology for Sustainable Environment

Production of Cellulase by *Aspergillus Sp.* Under Solid State Fermentation

Utharalakshmi N[1] and Ganesh Kumar A[2]*

[1]Department of Biotechnology, Sathyabama University, Chennai, Tamil Nadu, India
[2]National Institute of Ocean Technology, Chennai, Tamil Nadu, India

*Corres.author: microganesh@yahoo.com

Abstract : A new isolated from rhizosphere soil was optimized using solid state fermentation for increased cellulose production. The optimum cultural growth requirement were characterized at pH 6.0 and temperature 28 ºC for enhanced cellulase production. Six cellulolytic fungi were isolated, screened, maintained as pure culture. They were labeled as SB1, SB2, SB3, SB4, SB5 and SB6. The cultures were inoculated in rice bran and were maintained in incubator (28^0C±2). The cellulase activity was obtained on 0^{th}, 7^{th}, 14^{th}, 21^{st} and 28^{th} days. The maximum activity was obtained on 14^{th} day. The cellulase activity at different fermentation period was measured by filter paper assay. Amongst the isolated soil fungi SB1 produced the high concentration of reducing sugar 1.94 mg/ml and protein 0.87mg/ml respectively within short fermentation period.
Keywords: Cellulolytic fungi, Solid State Fermentation, *Aspergillus*, Cellulase

Introduction

Cellulases attained great importance industrially due to their applications in different raw material processing like animal feed production, brewing of fruit and vegetable juices, grain alcohol and starch processing[1, 2, 3]. Due to the minimal productivity and high cost submerged fermentation are unsatisfactory for industrial application[4]. Hence Solid state fermentation gained importance due to its cost effective method which has significantly low moisture content[5]. Broad cellulosic wastes are available naturally as agro waste like wheat bran, corn straw, paddy straw and rice bran to yield ethanol and biofuel. From the microbial flora effective bioconversions role can be played by bacteria, actinomycetes, fungi and few protozoa[6]. The current study involves in the isolation of novel cellulolytic fungi for obtaining the maximum enzyme yielding fungi (HE814595.1) in a short fermentation time.

Materials and Methods

Screening and Isolation of fungi

Soil is the potential natural environment for the isolation of promising cellulolytic fungi. The soil samples from decayed leave regions from different areas were collected in sterile plastic bags and were taken to laboratory under sterile condition for further screening procedures. Then, the samples were subjected to serial dilution and 10^{-5}, 10^{-6} and 10^{-7} were cultured on PDA plates and were maintained at 28°C for 5 days. Further pure cultures are maintained in slants at 4°C for future work. The culture plates were characterized based on their morphological and microscopical observation according to the standard procedures[7].

The Congo red clearing zone assay is the suitable qualitative method for screening the cellulase activity. The overnight incubated CMCA plates were observed with circular batches of isolated microorganisms and flooded with 0.1 percent Congo red solution then left for 15 min with intermittent shaking. Visible clear zone around the fungal growth was observed after washing with water and 1M NaCl solution[8].

Screening on CMC broth

1ml of fungal isolates were inoculated into the sterile CMC broth medium containing 0.2% (w/v) of 100ml in 250ml flask. The cultures were maintained within the shaker for 28 days at 28^0C. At periodical intervals of seven days the culture filterates were centrifuged at 6000 RPM for 15 min. supernatant were subjected to the estimation of enzyme and for reducing sugar to screen for fungi with high cellulase activity.

Cellulase Activity Assay

The collected crude filterate was quantified for cellulase protein by Lowry's method[9] and the quantity of reducing sugar liberated was decided by DNS method[10].

Fpase Assay

Filter paper enzyme activity (FPA) was determined using dinitro salicylic acid reagent for determination of reducing sugar, One unit (U) of enzyme activity is outlined because the amount of enzyme required to liberate one µmol of product per min. cellulase activity on SSF were analyzed at each periodical interval for the filterates[11].

Solid State Fermentation

The substrate rice bran was collected from the rice mills of Chennai and 0.3mm-1mm of particle size were selected for SSF. 30gm of rice bran was taken into a 250ml sterile container and also the moisture content was determined. The minimal medium (100 ml) containing trace components (10 ml) of composition given below was used. The minimal medium composition (g/L) includes 20.0 g of KH_2PO_4, 5.0 g of $MgSO_4$, and 1.0 g of $CaCl_2$. Trace elements of composition (g/L) 0.5 g of $MnSO_4$, 1.0 g of NaCl, 0.1 g of $FeSO_4$ $7H_2O$, 0.1 g of $CoCl_2$, 0.1 g of $ZnSO_4$ $7H_2O$, 0.1 g of $CuSO_4$ $5H_2O$ was prepared. From this 72ml was supplementary to the rice bran and autoclaved.[12]

Result and Discussion

Screening and Isolation

I n the present investigation six strains was isolated from the soil of decayed leaves region areas. The cellulase producing pure cultures were maintained on PDA slants at 4^0C. Previously 34 soil isolates were reported from the woody regions and cellulose plants resource areas 14 recorded as effective cellulolytic fungi[13]. The microscopic observation of sporulated spherical conidial head with the septal hyphae noted to be *Aspergillus sp.*[14]. Predominantly obtained cellulolytic fungi from the soil resource in spite of its woody, paper, agro wastes and alternative cellulosic content seems to be the often isolated and also the eminent producer for industrial cellulase production[14, 15, 16].

Determination of Cellulase Activity Assay

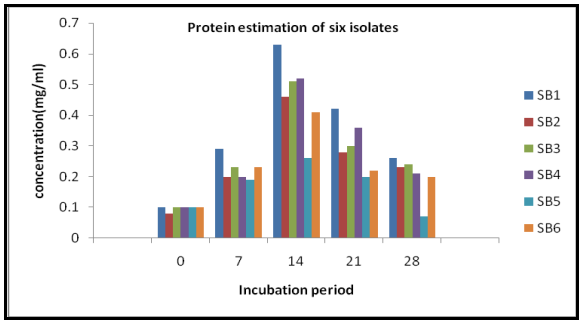

Figure 1. Fermentation period for Cellulase production obtained maximum at 14 day for SB1 which were very well influenced by the optimal pH 6 at 28^0C.

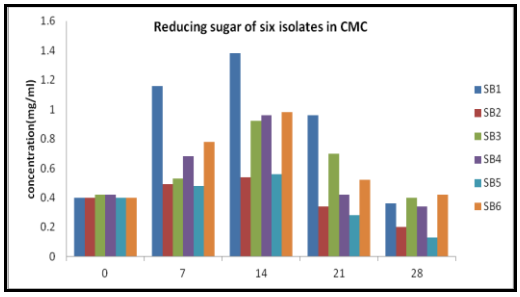

Figure 2. The influence of physical parameters for the total reducing sugar obtained within short fermentation time in SB1

Among the 6 isolates of SB1, SB2, SB3, SB4, SB5 and SB6. SB1 recorded to be the high cellulase producer relatively with the other 5 fungal isolates at the interval of seven days. The best conditions recorded was pH 6 at 28°C and yielded. SB1 produced 0.63mg/ml of enzyme and 1.38mg/ml of reducing sugar during the shortest fermentation period (Fig. 1 and Fig. 2). Greater numbers of *Aspergillus niger* isolated from the cotton industrial effluent discharged soil were reported to shown most activity of cellulase[17, 18] additionally reported best cultural conditions of comparable isolates from the spoiled coconut that yielded the maximum cellulase activity.

Solid State Fermentation

SSF of rice bran was investigated underneath the optimal condition of acidic pH 6 and best temperature at 28^0C yielded the utmost growth of with none inference. 10^8 reproductive spore suspensions were inoculated for its accelerating cellulase activity on the substrate because the higher concentration interferes with effectiveness of enzyme production[19]. Most promising parameters in SSF are pH scale, temperature and therefore the wetness demand for the active metabolism of fungi that impacts on the output of cellulase activity[12]. During this current study optimal condition of SSF were given the wetness content of 72ml/30g of rice bran that were maintained at 28^0C and pH 6 for 28 days .Optimal fermentation period recorded to be from the 7^{th}day which attain its maximum on 14^{th} day were quantitatively analyzed by determining the concentration of protein 0.87 mg/ml and reducing sugar of 1.94 mg/ml[20]. Yield of cellulase and its activity was 0.72 mg/ml in Fpase unit by SB1 occurred within short time that indicates SB1 fungus in future can create associate large role within the industrial application. Comparative production of protein, reducing sugar and Fpase on fermentation days was showed in.fig.3.This obtained fungal isolates state to the second leading cellulase producer therewith of *Trichoderma sp* that yields cellulase using SSF in vinegar industry effluents, corn straw, wheat bran, and rice straw were used because the substrates to serve for the cellulase producing[21].

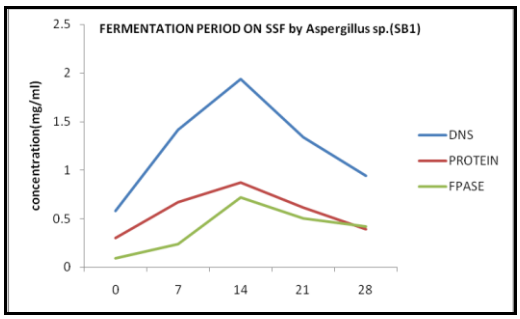

Figure 3. Fermentation period for maximum yield of cellulase production under SSF provided with optimized parameters.

Conclusion

The present investigation concludes that fungi SB1 submitted in European Institute of Bioinformatics got the accession number (HE814595.1) showed a greatest fermentation yielding point for enzyme from 7^{th}day that earned its peak at 14^{th}day. Overall enzyme produced on 14^{th} day was 0.87mg/ml that showed activity on cellulose and therefore the amount of reducing sugar recorded was 1.94mg/ml.

Acknowledgements

I am thankful to Sathyabama University for providing laboratory facilities and my colleagues for their motivated support.

References

1. Ogel, Z.B., Yarangumeli, K., Dundar, H., Ifrij, I., 2001. Submerged cultivation of *Scytalidium thermophilum* on complex lignocellulosic biomass for endoglucanase production. Enz. Microb. Technol. 28,689–695
2. Maryam Latifian, Zohreh Hamidi-Esfahani, Mohsen Barzeg, Evaluation of culture conditions for cellulase production by two *Trichoderma reese*i mutants under solid-state fermentation conditions. Bioresource Technology 98 (2007) 3634–3637
3. Sharada.R, Venkateswarlu.G, Narsi Reddy. M , Venkateshwar.S, Anand Rao.M Production of Cellulase by Solid State Fermentation International, Journal of Pharmaceutical Research and Development, 4(04): -2012 (224 – 230
4. Kang,S.W., Park,Y.S., Leee,J.S., Hong,S.I.,Kim,S.W., 2004. Production of cellulases and hemicellulases by *Aspergillus niger* KK2 from lignocellulosic biomass. Bioresource Technol., 91:153-156
5. Pandey, A, Solid state fermentation. Biochemical engineering, 2003,13: 81-84
6. Lynd, L.R., P.J. Weimer, W.H. van Zyl and I.S. Pretorius. (2002). Microbial cellulose utilization: Fundamentals and biotechnology. *Microbiol. Mol. Biol. Rev*., 66: 506-577
7. Domsch K.H., Gams W., Anderson T.H., 1980. Compendium of soil fungi, Vol.1Academic press, London
8. Ponnambalam AS, Deepthi RS , Ghosh AR. Qualitative display and measurement of enzyme activity of isolated cellulolytic bacteria. Biotechnol. Bioinf. Bioeng. 2011, 1(1):33-37
9. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. Protein measurement with folin phenol reagent .Journal of Biological Chemistry. 1951; 193:265-275.
10. Miller GL. Use of dinitro Salicylic acid reagent for determination of reducing sugar, Biotechnology Bioengineering Symposium. 1972; 5:193-219.
11. Ghose, T.K., 1987. Measurements of cellulase activities. Pure Appl. Chem. 59, 257–268.
12. Ganesh Kumar.A, Sekaran.G , Sarayu Krishnamoorthy. Solid state fermentation of *Achras zapota* lignocelluloses by *Phanerochaete chrysosporium*. Bioresource Technology 97 (2006) 521–1528
13. Shaikh N.M. Patel A.A., Mehta S.A., Patel N.D. Isolation and screening of cellulolytic bacteria inhabiting different environment and optimization of cellulase production. Universal Journal of Environmental Research and Technology. 2013, 3(1), 39-49.
14. .Soma Mrudula and Rangaswamy Murugammal. Production of Cellulase by *Aspergillus niger* under submerged and solid state fermentation using coir waste as a substrate. Brazilian Journal of Microbiology,2011, 42, 1119-1127.
15. Sri Lakshmi A,. Narasimha G. Production of cellulases by fungal cultures isolated from forest litter soil. Ann.For.Res. 55(1) 2012.
16. Shobana P. and. Uma Maheswari N. Production of Cellulase from *Aspergillus fumigatus* under Submerged And Solid State Fermentation Using Agricultural Waste. International Journal of Advances, Pharmacy, Biology and Chemistry 2013. 2(4), 595-598.
17. Narasimha G., Babu G.V.A.K., Rajasekhar Reddy B., 1998. Cellulolytic activity of fungal cultures isolated from soil contaminated with effluents of cotton ginning industry. J. Scientific & Industrial Research.57: 617-620.
18. .Siva Sakthi.S.,Saranraj.P and Rajasekar.M. Optimization for cellulase production by *Aspergillus niger* using paddy straw as substrate. International journal of advanced scientific and technical research.2011. 1(1),69-85.
19. Sharada.R,Venkateswarlu.G, Narsi Reddy. M , Venkateshwar.S, Anand Rao.M. Production of Cellulase by solid state fermentation. International Journal of Pharmaceutical Research and Development. 4(04), 224-230, 2011
20. Sadaf Jahangeer, Nazia Khan, Saman Jahangeer, Muhammad Sohali, Saleem Shahzad, Aqeel Ahmad & Shakeel Ahmed Khan., 2005. Screening and characterization of fungal cellulases isolated from the native environmental source. Pak. J. Bot., 37(3): 739-748.
21. Jian Liu and Jichu Yang. Cellulase production by *Trichoderma koningii* AS3.4262 in solid state fermentation using lignocellulosic waste from the vinegar industry. Food technol.Biotechnol.45(4): 420-425.

*****